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Effects of purines on the longitudinal muscle of the rat colon

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1 Adenosine and adenosine 5'-triphosphate (ATP) have been reported to cause relaxation of the rat colon longitudinal muscle preparation; the purinoceptors mediating this effect were investigated by use of a series of agonists and antagonists.

2 The tissue was precontracted with carbachol (1 μM), and the purines induced reversible relaxations with a potency order of 5'-N-ethylcarboxamidoadenosine (NECA) >N^6-cyclopentyladenosine (CPA)=adenosine 5'-(α,β-methylene) triphosphonate (AMPCPP) >adenosine=adenylyl 5'-(β,γ-methylene) disphosphonate (AMPPCP)=ATP. The P_1-selective antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (3 μM) shifted to the right the log concentration-response curves of all these agonists except for AMPCPP, indicating that they all act via P_1-purinoceptors. The order of potency of the adenosine analogues and the relatively high concentrations of the antagonist required indicated that these receptors are of the A_2 subtype. The P_2-selective antagonist suramin (300 μM) inhibited responses to AMPCPP, but not to the other agonists.

3 The dephosphorylation of the nucleotides was studied by high performance liquid chromatography following incubation with the longitudinal muscle preparation for up to 30 min. ATP was rapidly degraded, largely to adenosine, and AMPPCP and AMPCPP were also degraded, although more slowly, to adenosine and adenosine 5'-(α,β-methylene) diphosphonate (AMPCP) respectively. AMPCP, like AMPCPP, caused relaxations by acting on P_2-purinoceptors, as it was also inhibited by suramin (300 μM). Incubation of the tissue with adenosine deaminase abolished responses to adenosine, reduced those to ATP and AMPPCP, but had no effect on those to AMPCPP. ATP and AMPPCP therefore appear to be acting on the A_2 receptors in this tissue largely via their degradation product adenosine.

4 The longitudinal muscle of the rat colon therefore contains both P_1- and P_2-purinoceptors, which both mediate relaxation. The P_1-purinoceptors are of the A_2 subtype and the P_2-purinoceptors are probably of the P_{2Y} subtype, although the rapid degradation of the nucleotides means that it is difficult to classify them with certainty.

Keywords: Rat colon longitudinal muscle; purinoceptors; ATP; adenosine; ectonucleotidases; suramin

Introduction

The purinoceptors which mediate the many and varied pharmacological responses to adenosine and adenine nucleotides have been clearly subdivided into P_1, recognising adenosine, and P_2, recognising adenosine 5'-triphosphate (ATP) (Burnstock, 1978), and selective antagonists for each of these receptor types exist. Xanthine derivatives such as theophylline and its analogues are P_1-purinoceptor antagonists, having no effect on the actions of ATP in most tissues, and the trypanocidal drug suramin has recently been shown to act as a P_2-purinoceptor antagonist in a number of tissues (Dunn & Blakely, 1988; Stone, 1989; Den Hertog *et al.*, 1989a; b; Hoyle *et al.*, 1990; Burnstock, 1990; Kennedy, 1990; Leff *et al.*, 1990; Bailey *et al.*, 1992). As well as acting at P_2-purinoceptors, ATP is rapidly and sequentially dephosphorylated by ectonucleotidases present on the surface of cells, ultimately to adenosine, which is more slowly taken up into cells or deaminated to the inactive inosine (Pearson & Slakey, 1990). This transformation of ATP into adenosine may affect the observed responses to ATP, as not only will it reduce its effects at P_2-purinoceptors but it may also result in indirect effects at P_1-purinoceptors. Unfortunately, no selective inhibitors of this breakdown are available (Hourani & Chown, 1989), but the contribution of adenosine to the effects of ATP can be investigated using adenosine deaminase (ADA) to remove any adenosine produced, or inhibitors of adenosine uptake such as S-(4-nitrobenzyl)-6-thioguanosine (NBTG) to enhance the effects of adenosine.

P_1-purinoceptors have been subdivided into A_1 and A_2, and these can be distinguished in functional studies by the order of potency of adenosine analogues, with N^6-substituted compounds such as N^6-cyclopentyladenosine (CPA) being more potent than 5'-substituted compounds such as 5'-N-ethylcarboxamidoadenosine (NECA) on A_1 receptors, but less potent on A_2 receptors. In addition, the antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) has nanomolar affinity for A_1 receptors and micromolar affinity for A_2 receptors, and clearly distinguishes between the two receptor subtypes in isolated tissue preparations (Collis *et al.*, 1989; Collis, 1990; Bruns, 1990; Jacobson, 1990). In general, A_2 receptors have been found to mediate the inhibitory effects of adenosine on smooth muscle, causing either relaxation of precontracted muscle or reduction in the contractile effects of other agonists, whereas A_1 receptors mediate contractile effects and the presynaptic inhibition of transmitter release (White, 1988; Olsson & Pearson, 1990).

P_2-purinoceptors on smooth muscle have also been subdivided into P_{2X} and P_{2Y} (Burnstock & Kennedy, 1985; Gordon, 1986) and these may be distinguished by the use of ATP analogues (Cusack & Hourani, 1990), although this subdivision is not as firmly based as that for P_1-purinoceptors as no selective competitive antagonists exist, suramin being equally effective on each subtype (Dunn & Blakely, 1988; Den Hertog *et al.*, 1989a; b; Hoyle *et al.*, 1990; Leff *et al.*, 1990). On P_{2Y} receptors, 2-substituted analogues such as 2-methylthioadenosine 5'-triphosphate (2-MeSATP) are more potent than ATP which is more potent than its methylene phosphonate analogues such as adenosine 5'-(α,β-methylene) triphosphonate (AMPCPP) and adenylyl 5'-(β,γ-methylene)

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diphosphonate (AMPPCP), whereas on P_{2X} receptors AMPPCP and AMPCPP are more potent than ATP which is equipotent with 2-MeSATP (Burnstock & Kennedy, 1985). Although the structure-activity relationships for ATP and its analogues in some tissues may be affected by the rapid degradation of ATP and its 2-substituted analogues and the relative stability of the methylene phosphonate analogues (Welford *et al.*, 1986; 1987), the different structure-activity relationships for P_{2X} and P_{2Y} receptors have been confirmed by use of a series of stable isopolar phosphonate ATP analogues (Cusack *et al.*, 1987). In addition, two nucleotide analogues, L-adenylyl 5'-(β,γ-methylene) diphosphonate (L-AMPPCP) and adenosine 5'-(2-fluorodiphosphate) (ADP-β-F) have been developed as selective agonists for P_{2X} and P_{2Y} receptors respectively (Hourani *et al.*, 1986; 1988). Although in general, P_{2X} receptors mediate contraction of smooth muscle and P_{2Y} receptors mediate relaxation and this was a basis for the original subdivision (Burnstock & Kennedy, 1985), this is not a secure criterion for receptor classification and indeed in two tissues, neonatal rat duodenum and the rat colon muscularis mucosae, ATP induces contraction not relaxation via receptors whose structure-activity relationships are indicative of the P_{2Y} subtype (Nicholls *et al.*, 1990; Bailey & Hourani, 1990).

A question which has been a matter of continuing controversy is the extent to which ATP may interact directly with P_1 receptors (Stone, 1989; Bruns, 1990). There have been a number of reports of the effects of ATP being blocked by P_1 antagonists in some tissues, although it is not always clear whether this is a direct effect of ATP or due to its degradation to adenosine. In some cases the more stable analogue AMPPCP has also been shown to have P_1 effects, which has generally been interpreted as indicating that the P_1 effects of ATP itself are direct, not via its breakdown to adenosine (Dahlen & Hedqvist, 1980; Collis & Pettinger, 1982; Wiklund *et al.*, 1985), although it has also been taken as evidence that AMPPCP is itself degraded (Moody & Burnstock, 1982), or that a third class of purinoceptor, called P_3, exists at which both adenosine and adenine nucleotides are active (Shinozuka *et al.*, 1988). This controversy may be resolved by our recent finding that in some tissues AMPPCP itself can have direct effects on P_1-purinoceptors whereas ATP, 2-MeSATP and AMPCPP do not, in spite of the fact that ATP is much more rapidly degraded to adenosine than is AMPPCP. In the rat colon muscularis mucosae and the rat duodenum, AMPPCP appears to act almost entirely via P_1 receptors rather than by the P_{2Y} receptors which are also present, as its effects can be completely inhibited by the P_1 antagonist, 8-sulphophenyltheophylline (8-SPT) in the same way as those of adenosine, whereas in the guinea-pig taenia caeci, 8-SPT causes only a roughly two fold shift in the concentration-response curve to AMPPCP, indicating that it acts largely via the P_{2Y} receptors here (Bailey & Hourani, 1990; Hourani *et al.*, 1991). Somewhat different pA_2 values were obtained for 8-SPT against AMPPCP in these tissues (approximately 5 in the taenia caeci, approximately 6 in the duodenum and colon), indicating that different P_1 receptors might be present, and that AMPPCP might have selectivity for A_1 receptors. However, as 8-SPT has been reported not to be selective for A_1 receptors in isolated tissues (Collis *et al.*, 1987) although it does show some selectivity in binding studies (Bruns *et al.*, 1986), these small differences were not easy to interpret. Another problem is that the affinity of P_1 receptors for agonists and antagonists is known to be dependent on the species used, with rat tissues in general having a higher affinity than guinea-pig tissues (Collis, 1990), which could also explain the differences we observed with 8-SPT. However, using a series of agonists and the highly A_1-selective antagonist DPCPX we have shown that on the rat colon muscularis mucosae the P_1 receptors are of the A_1 subtype whereas those on the rat duodenum are a mixture of A_1 and A_2, with AMPPCP apparently acting on the A_1 population although adenosine acts on the A_2 (Nicholls *et al.*, 1992; Bailey *et al.*, 1992). As the P_1 receptors on the taenia caeci are known to be of the A_2 subtype (Burnstock *et al.*, 1984), this would be consistent with the hypothesis that AMPPCP has selectivity for A_1 receptors, although species differences could also play a role. In addition, in the rat urinary bladder in which AMPPCP causes contractions via P_{2X} receptors, its effects were not enhanced by a concentration of DPCPX which inhibited the effect of adenosine, suggesting that it had no action on the inhibitory A_2 receptors present in this tissue (Nicholls *et al.*, 1992).

To clarify this matter we wished to use a rat tissue similar to the guinea-pig taenia caeci, which relaxes to purines via P_{2Y} and A_2 receptors, and to investigate whether AMPPCP would have any P_1 effect in this tissue. Rather than use a vascular tissue such as the rat aorta in which there is the complication that any observed relaxation to adenosine or to ATP may be indirect, via receptors on the endothelium rather than on the smooth muscle (Rose'Meyer & Hope, 1990; Olsson & Pearson, 1990), we chose to investigate the longitudinal muscle of the rat colon. This tissue has not been widely used but has been reported to relax in response to adenosine and to ATP when precontracted with the muscarinic antagonist oxotremorine (Romano, 1981), and therefore appeared likely to be pharmacologically equivalent to the guinea-pig taenia caeci.

Methods

Pharmacological studies

Male Wistar rats (150–250 g) were killed by cervical dislocation and the distal colon removed and placed in warm (32°C) Tyrode buffer (ionic composition (mM): Na^+ 149.1, K^+ 2.8, Ca^{2+} 1.8, Mg^{2+} 2.1, Cl^- 147.5, $H_2PO_4^-$ 0.3, HCO_3^- 11.9, glucose 5.6) pregassed with 95%O_2/5% CO_2. The dissection of the longitudinal muscle of the colon was carried out as described by Romano (1981) and Bailey & Jordan (1984), with minor modifications. Briefly, a glass pipette, external diameter 5 mm, was placed inside the colon and the outer tubular layer of longitudinal muscle was removed by gentle rubbing with wet cotton wool, leaving a thick walled tube, the muscularis mucosae. The longitudinal muscle was suspended in a 3 ml organ bath at 32°C in gassed Tyrode solution, and contractions were recorded isometrically under a resting tension of 1 g with a Grass FT03 strain gauge and displayed on a Grass 79C polygraph. The tone of the tissue was raised with carbachol (1 μM for 3 min, which caused 50–70% of the maximal contraction) and relaxations to adenosine and adenine nucleotides were expressed as % relaxation of this carbachol-induced contraction. The longitudinal muscle was allowed to equilibrate for 90 min before control concentration-response curves to purinoceptor agonists were determined, followed by incubation with an antagonist, ADA or NBTG for 40 min before the concentration-response curves were repeated in the presence of these substances. Recovery of responses to the agonists was established following washout of these substances for up to 40 min; 12–20 min were allowed between doses of the purinoceptor agonists, which were left in contact with the tissue for 2–5 min until a maximal relaxation had been observed.

Degradation studies

Segments of rat colon longitudinal muscle (approximately 30 mg wet weight) were suspended in 3 ml organ baths as described for the pharmacological studies. Following preincubation for 90–120 min with frequent washing, each was exposed to ATP, AMPPCP and AMPCPP (100 μM at 30 min intervals), and samples of the bathing medium were taken at 2, 5, 10 and 20 min and frozen for later analysis by high performance liquid chromatography as described by Welford *et al.* (1986, 1987).

Materials

ATP, AMPPCP, AMPCPP, AMPCP, adenosine, ADA (Type VI) and NBTG were obtained from Sigma UK. Ltd, DPCPX, 2-MeSATP, CPA and NECA were obtained from Research Biochemicals, and suramin was a generous gift from Bayer, UK. Other purine analogues were kindly provided by Dr Noel J. Cusack, Whitby Research Inc, Richmond, Virginia, U.S.A. CPA (10 mM) was dissolved in 20% ethanol, DPCPX (1 mM) was dissolved in 2% aqueous dimethylsulphoxide (DMSO) containing 6 mM NaOH and NBTG (50 mM) was dissolved in DMSO. After dilution corresponding to the final bath concentration of the substances used these solvents had no effect on the responses of the tissue. The ADA solution was supplied in 50% glycerol-0.01 M potassium phosphate and was diluted in distilled water to give a stock solution of 200 units ml^{-1}.

Results

Adenosine, ATP, AMPPCP, AMPCPP, NECA and CPA each relaxed the carbachol-contracted rat colon longitudinal muscle, and the order of potency was NECA> CPA = AMPCPP> adenosine = AMPPCP = ATP (Figure 1). DPCPX

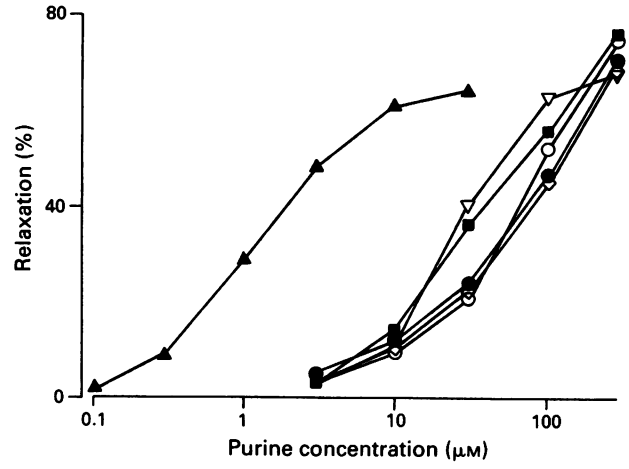

Figure 1 Relaxation of the rat colon longitudinal muscle by adenosine (●), NECA (▲), CPA (■), ATP (○), AMPPCP (◇) or AMPCPP (▽). Each point is the mean of at least 15 determinations, and the error bars, which never exceeded 5%, have been omitted for clarity. For abbreviations, see text.

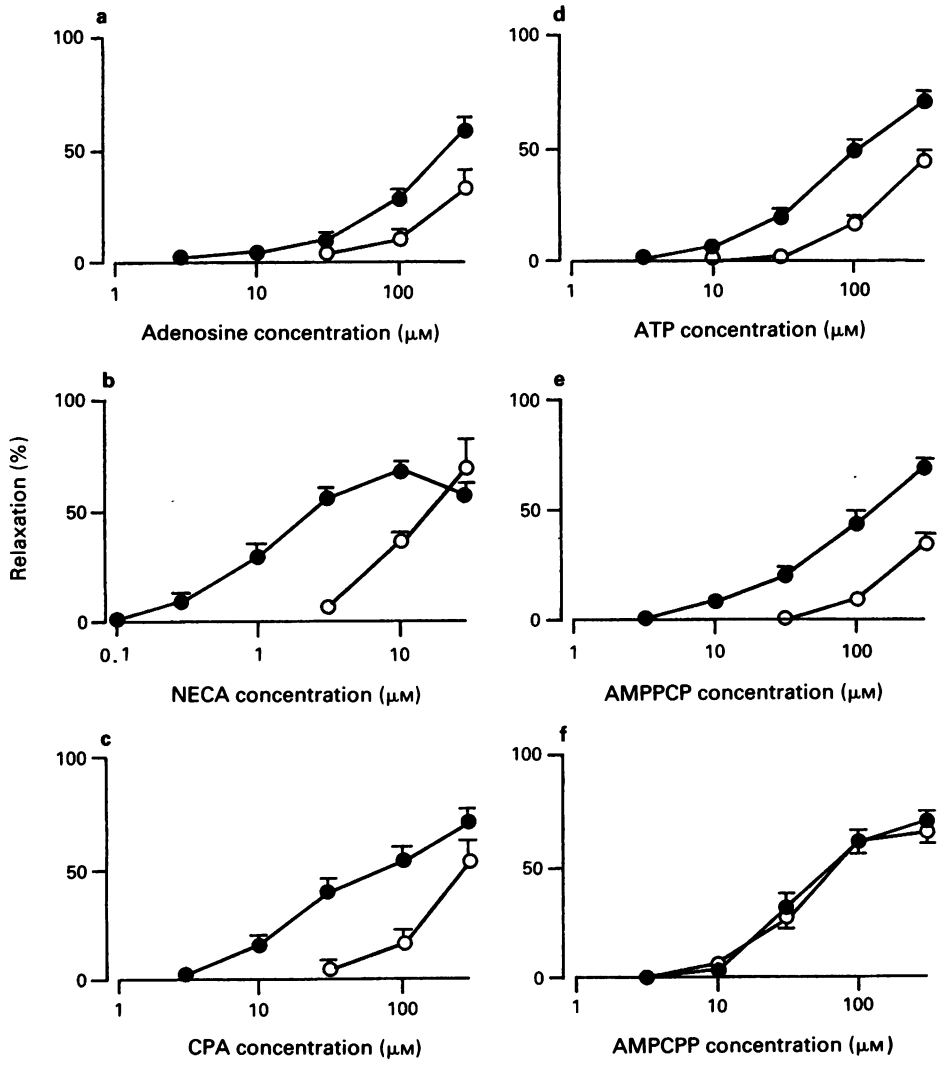

Figure 2 Relaxation of the rat colon longitudinal muscle by (a) adenosine, (b) NECA, (c) CPA, (d) ATP, (e) AMPPCP or (f) AMPCPP alone (●) or in the presence of DPCPX (3 μM) (○). Each point is the mean of at least 4 determinations and the vertical bars show s.e.mean. For abbreviations, see text.

(3 μM) inhibited the responses to NECA, CPA, adenosine, AMPPCP and ATP, but did not affect the relaxations induced by AMPCPP (Figure 2), whereas suramin (300 μM) inhibited the responses to AMPCPP but not those of the other agonists (Figure 3). DPCPX (1 μM) caused smaller shifts to the right of the log concentration-response curves to all the agonists except for AMPCPP (results not shown), but Schild analysis was not performed as concentrations of DPCPX above 3 μM could not be achieved. L-AMPPCP (100 μM) and 2-MeSATP (100 μM) were almost inactive, ADP-β-F(100 μM) induced a relaxation similar to that of ATP (100 μM), while adenosine 5'-(α,β-methylene) diphosphonate (AMPCP) (100 μM) induced a relaxation similar to that of AMPCPP (100 μM) (Figure 4). Representative traces showing the relaxations induced by the agonists are shown in Figure 4. The responses to AMPCP were inhibited by suramin (300 μM) in a similar manner to those of AMPCPP (results not shown).

Incubation of the tissues with ADA (2 units ml^{-1}) abolished the relaxations induced by adenosine and inhibited those induced by ATP and AMPPCP, but had no effect on those induced by AMPCPP (Figure 5). Incubation of the tissues with NBTG (50 μM) potentiated responses to adenosine, causing a roughly three fold shift to the left of the log concentration-response curve, and also potentiated responses to AMPCPP but to a somewhat lesser extent (results not shown).

Degradation studies

Dephosphorylation of ATP by the rat longitudinal muscle preparation was rapid, with approximately 80% being degraded, largely to adenosine and AMP, during 30 min incubation. AMPPCP was degraded more slowly to adenosine, with approximately 20% conversion during 30 min incubation, and AMPCPP was degraded to AMPCP, with approximately 30% conversion over 30 min (Figure 6).

Discussion

These results show that the outer longitudinal muscle layer of the rat colon contains P_1- and P_2-purinoceptors which both mediate relaxation, and that the responses of this tissue to purines are qualitatively similar to the responses seen in the guinea-pig taenia caeci, the longitudinal muscle of the guinea-pig caecum. For the P_1-purinoceptor in the longitudinal muscle the potency order of agonists is NECA> CPA> adenosine, and DPCPX has an apparent dissociation constant in the micromolar range, suggesting that the receptor is of the A_2 subtype (Bruns, 1990; Collis, 1990; Daly, 1990). DPCPX (3 μM) caused a somewhat smaller shift to the right of the log concentration-response curve to adenosine compared with NECA and CPA, which may suggest that it also interfered with adenosine uptake. In general the post-

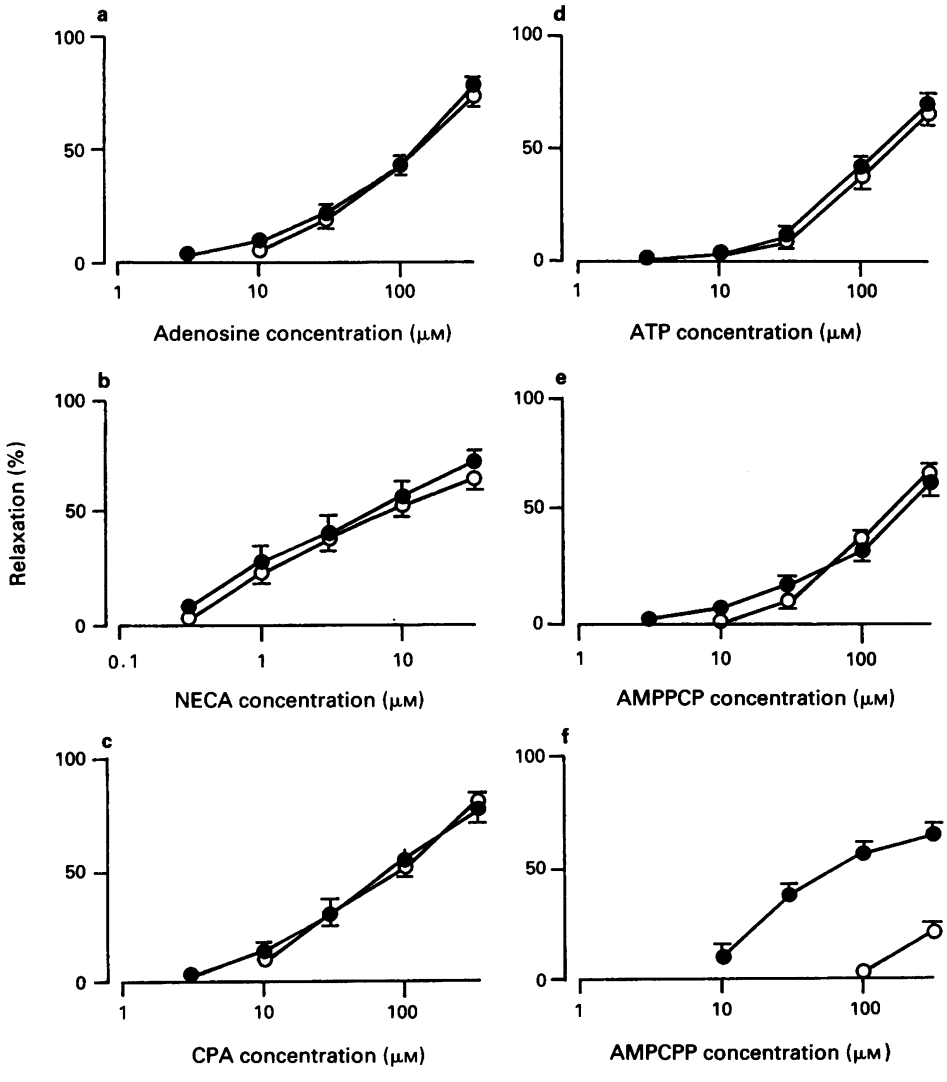

Figure 3 Relaxation of the rat colon longitudinal muscle by (a) adenosine, (b) NECA, (c) CPA, (d) ATP, (e) AMPPCP or (f) AMPCPP alone (●) or in the presence of suramin (300 μM) (○). Each point is the mean of at least 4 determinations and the vertical bars show s.e.mean. For abbreviations, see text.

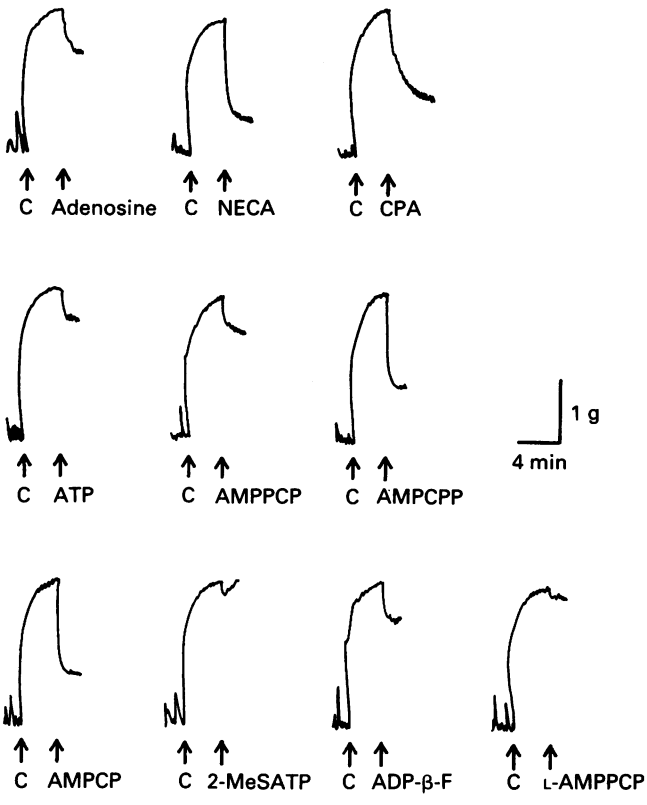

Figure 4 Representative traces showing relaxations of the rat colon longitudinal muscle to purines after precontraction of the tissue with carbachol (1 μM) (C). The concentration of purine added in each case was 100 μM, except for NECA which was 10 μM. For abbreviations used in figure, see text.

synaptic inhibitory effects of adenosine in smooth muscle including the guinea-pig taenia caeci are mediated by A_2 receptors, so the finding that the adenosine receptor here is of the A_2 subtype is consistent with results in other tissues (White, 1988; Stone, 1989; Kennedy, 1990; Olsson & Pearson, 1990). In those tissues in which adenosine causes contraction, such as the renal vasculature (Kenakin & Pike, 1987) and the guinea-pig myometrium (Smith *et al.*, 1988), this appears to be via A_1 receptors, and this is also the case in the rat colon muscularis mucosae (Bailey *et al.*, 1992). It has also been reported that the guinea-pig aorta and trachea each possess both A_1 and A_2 receptors which mediate contraction and relaxation respectively, although for adenosine the A_2-mediated relaxation is dominant (Farmer *et al.*, 1988; Stoggall & Shaw, 1990). The existence in the rat colon of excitatory A_1 receptors (on the muscularis mucosae) and inhibitory A_2 receptors (on the longitudinal muscle) is therefore consistent with this general pattern, although the two receptor types are clearly at anatomically distinct locations within the colon, a possibility which has not been investigated in the other tissues.

The rat colon longitudinal muscle also possesses P_2-purinoceptors, as AMPCPP caused a relaxation which was inhibited by the P_2-purinoceptor antagonist suramin but not by DPCPX. The actions of ATP and AMPPCP appeared however to be mediated via the A_2 receptor, as they were not inhibited by suramin but were inhibited by DPCPX at the same concentration as was adenosine. It seems likely that this A_2 effect was at least partially indirect and a result of the degradation of ATP and AMPPCP to adenosine, as the responses to ATP and AMPPCP were reduced by ADA, which had no effect on the responses to AMPCPP. The adenosine uptake inhibitor NBTG potentiated responses to adenosine as expected, but also potentiated responses to AMPCPP which does not act on A_2 receptors here, and therefore was not useful for resolving the question as to whether the actions of ATP and AMPPCP were direct or via adenosine. The potentiation by NBTG of responses to AMP-

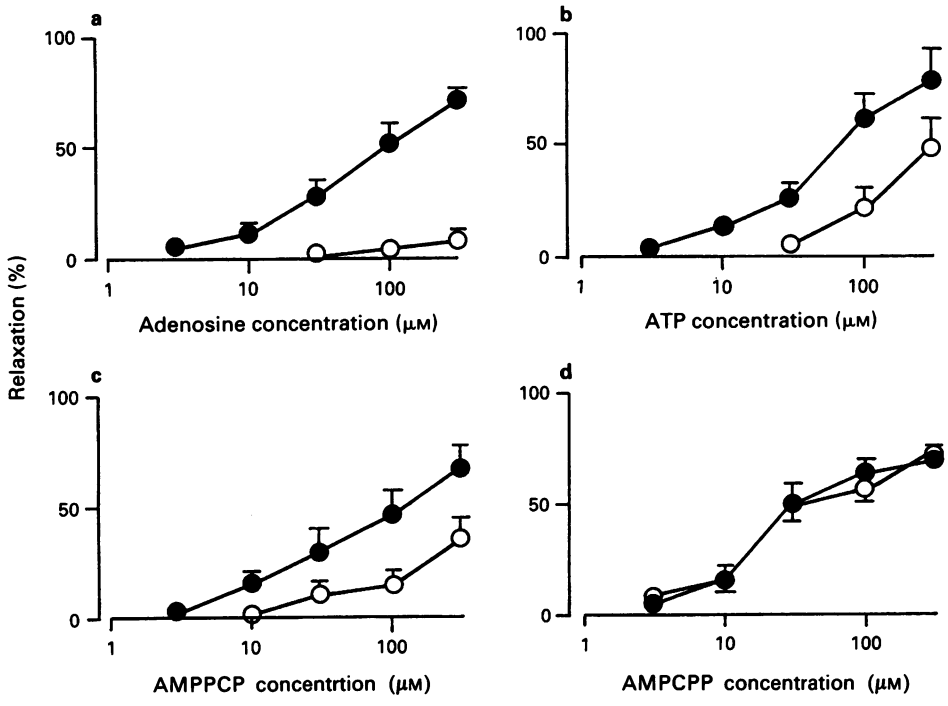

Figure 5 Relaxation of the rat colon longitudinal muscle by (a) adenosine, (b) ATP, (c) AMPPCP or (d) AMPCPP alone (●) or in the presence of adenosine deaminase (2 units ml^{-1}) (○). Each point is the mean of at least 5 determinations and the vertical bars show s.e.mean. For abbreviations, see text.

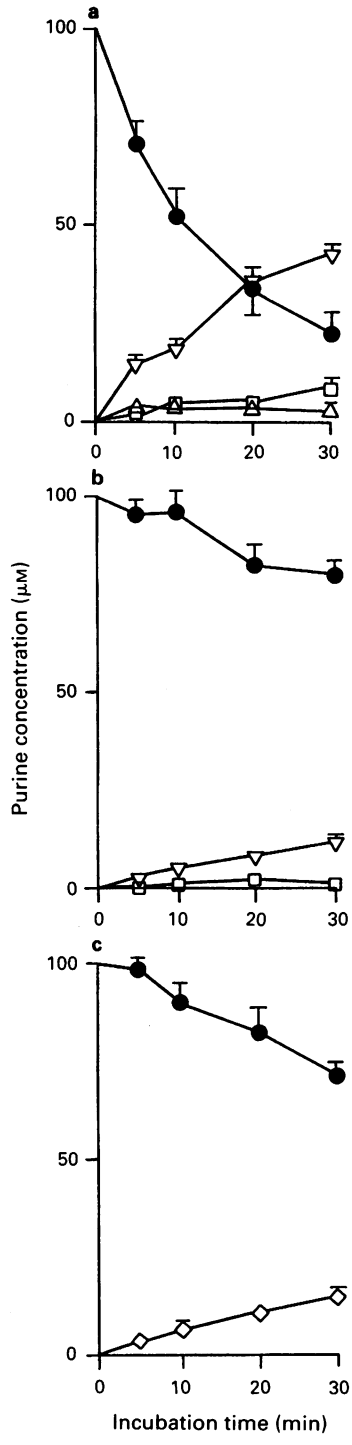

Figure 6 Degradation of purines by the rat colon longitudinal muscle preparation. Removal of (a) ATP, (b) AMPPCP or (c) AMP-CPP (●) and appearance of ADP (△), AMP (□), adenosine (▽) or AMPCP (◇). Each point is the mean of at least 4 determinations and the vertical bars show s.e. mean. For abbreviations, see text.

CPP was presumably due to increased endogenous adenosine levels enhancing all relaxant responses, but this was not investigated further. It is probable that AMPCPP retained its P_2-purinoceptor activity even though it is also significantly degraded, to at least the same extent as AMPPCP, because its breakdown product was not adenosine but AMPCP, which was inhibited by suramin and therefore acts on P_2-purinoceptors. These findings are in contrast to our results with the rat colon muscularis mucosae and the rat duodenum, where it is clear that despite its rapid degradation, ATP has little if any P_1-purinoceptor activity whereas AMPPCP acts almost entirely and directly via P_1-purinoceptors (Bailey & Hourani, 1990; Hourani *et al.*, 1991). Unfortunately the uncertainty regarding the direct A_2 receptor activity of AMPPCP itself in the longitudinal muscle means that the use of this tissue has not resolved our initial hypothesis that AMPPCP might have A_1-selective P_1-purinoceptor activity, as had been suggested by our previous studies (Bailey & Hourani, 1990; Nicholls *et al.*, 1990; Hourani *et al.*, 1991; Bailey *et al.*, 1992; Nicholls *et al.*, 1992).

Although the P_2-purinoceptor which mediates relaxation in the guinea-pig taenia caeci is clearly of the P_{2Y} subtype, with an order of potency of 2-MeSATP>ATP>AMPCPP (Burnstock *et al.*, 1984; Burnstock & Kennedy, 1985), the P_2-purinoceptor in the rat colon longitudinal muscle is not so easy to define by use of these agonists as ATP itself had no P_2-purinoceptor activity in this tissue and 2-MeSATP was almost inactive. The weak activity of 2-MeSATP may be due to its rapid breakdown to 2-methylthioadenosine, which is somewhat less active than adenosine on the A_2 receptors in the guinea-pig taenia caeci (Satchell & Maguire, 1975) and is therefore likely to be less active in the longitudinal muscle preparation. Indeed the very rapid degradation of the nucleotides, including the normally resistant AMPPCP and AMPCPP, by this preparation makes analysis of the structure-activity relationships of the P_2-purinoceptor very difficult. The response mediated by this receptor is a relaxation which is qualitatively similar to that seen in the guinea-pig taenia caeci, which might suggest involvement of a P_{2Y}-purinoceptor, but this is not a reliable method for receptor classification, particularly in view of the existence of P_{2Y}-purinoceptors which mediate contraction on the muscularis mucosae and the neonatal rat duodenum (Bailey & Hourani, 1990; Nicholls *et al.*, 1990). The lack of activity of L-AMPPCP and the activity of ADP-β-F in the longitudinal muscle would appear to confirm that the P_2-purinoceptor here is of the P_{2Y} subtype, as L-AMPPCP and ADP-β-F are selective agonists at P_{2X}- and P_{2Y}-purinoceptors respectively (Hourani *et al.*, 1986; 1988). However, the same argument could in theory apply to L-AMPPCP as to 2-MeSATP, as its potential breakdown product, L-adenosine, is inactive on P_1-purinoceptors (Brown *et al.*, 1982), and similarly ADP-β-F could be acting via its probable breakdown product, adenosine, so this evidence cannot be regarded as conclusive. What is needed, both to define the P_2-purinoceptor in this tissue and to resolve the question of whether ATP and AMPPCP act directly or indirectly on the P_1-purinoceptor, is a selective inhibitor of the ectonucleotidases which are responsible for the breakdown.

Notwithstanding these uncertainties, it is clear from the work presented here together with our previous work (Bailey & Hourani, 1990; Bailey *et al.*, 1992), that the two preparations from the rat colon, the muscularis mucosae and the longitudinal muscle, have different P_1-purinoceptor subtypes, A_1 mediating contraction and A_2 mediating relaxation, and that they also possess P_2 purinoceptors which mediate the same effect in each case as the P_1 purinoceptors. The physiological significance of these receptors is unknown, but probably reflects the widespread roles of purines as transmitters and modulators in the autonomic nervous system. The nature of the P_2-purinoceptor on the longitudinal muscle is still undefined, but it is likely to be of the P_{2Y} subtype as in the muscularis mucosae, although the response observed is different in the two preparations. In the longitudinal muscle preparation the breakdown of nucleotides is extremely rapid, and is considerably faster than that previously observed in the muscularis mucosae in spite of the fact that the longitudinal muscle is a much smaller tissue, with a wet weight of about 30 mg compared to around 200 mg for the muscularis mucosae. This very rapid degradation undoubtedly affects the responses to the nucleotides, and this makes the study of the pharmacology of the P_2-purinoceptors in this

tissue somewhat problematical, and shows clearly the care which must be taken when attempting to use nucleotide agonist potencies to define receptor subtypes.

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